Chapter 5

HPLC conditions are critical for the detection of GABA by microdialysis

Abstract

In microdialysis studies, neither exocytotic release of GABA, nor the presence of $GABA_B$ autoreceptors, have been clearly established. It was investigated whether the chromatographic separation of GABA may have contributed to discrepancies in the literature. After extending the profile of the HPLC chromatogram to a retention time of 60 minutes, it was observed that various unknown compounds of biological origin co-eluted near the GABA peak. The retention time of GABA appeared to be extremely sensitive to pH; even at a retention time of around 60 min there was only a small pH window (5.26 ± 0.01) where GABA was consistently well separated from co-eluting compounds.

GABA determined by the improved assay was sensitive to TTX, calcium depletion and the $GABA_B$ autoreceptor agonist baclofen.

The present results illustrate that if the proper analytical conditions are applied, extracellular GABA can be sampled and quantitated by microdialysis in free-moving animals. However, when the time-curves are considered, there is a striking delay of about 15-30 min before the effects of TTX, calcium depletion or baclofen are observed, as compared to the reported response of neurotransmitters such as dopamine (less than 5 min). It is assumed that the glial cells serve as a buffer between the GABA synapse and the microdialysis probes. It is proposed that microdialysis samples measure synaptic GABA indirectly, through glial cells surrounding the synapses.

Introduction

Gamma-aminobutyric acid (GABA) is the principal predominant inhibitory neurotransmitter in the brain. It is heterogeneously distributed throughout the brain and regulates many neuronal processes. Brain GABA content is greater than most other neurotransmitters, and is found mostly in spiny or aspiny granular neuronal cells (Jones & Mogenson, 1980a, b; Walaas & Fonnum, 1979; Bolam *et al,* 1983), interneurons (McCormick *et al,* 1993) and in glial cells such as astrocytes (Fraser *et al,* 1994).

Due to the various biological roles of GABA, and its involvement in so many neurological conditions, many researchers have performed microdialysis studies to determine the concentration of GABA in extracellular space. Discrepancies have been reported by different investigators, regarding basal extracellular GABA levels in the same brain regions (Biggs *et al,* 1992; Rakovska *et al,* 1998; Rowley *et al,* 1995), and also the effects of various pharmacological agents on these levels (Timmerman & Westerink, 1997). Calcium omission and TTX infusion have been reported to be partly effective (Campbell *et al,* 1993; Singewald *et al,* 1993; Rakovska *et al,* 1998; Osborne *et al,* 1990; Drew & Ungerstedt, 1991), slightly effective (Xi & Kalivas, 2003; Stengard & O'Connor, 1994; Timmerman & Westerink, 1997), and non-responsive (Drew *et al,* 1990; Timmerman & Westerink, 1995; Morari *et al,* 1993; Ferraro & O'Connor, 2000) in reducing basal GABA levels.

Also, studies that reported TTX sensitivity, observed a very slow decrease in basal GABA levels which only reached statistical significance after 30 minutes or more, as compared to classical neurotransmitters such as dopamine (Feenstra & Botterblom, 1996; Osborne *et al,* 1990; Bourdelais & Kalivas, 1992; Smith & Sharp, 1994; Stengard & O'Connor, 1994; Bourdelais & Deutsch, 1994; Timmerman and Westerink, 1997).

The presence of $GABA_B$ autoreceptors in the brain is well established. *In vitro* reports have demonstrated that a significant decrease in basal GABA output is seen with the addition of the $GABA_B$ agonist baclofen (Lambert & Wilson, 1994; Lanza *et al,* 1993; Baumann *et al,* 1990, Waldmeier *et al,* 1988). However in microdialysis studies, administration of baclofen is reported to have little

(Bourdelais & Kalivas, 1992), or no effect (Richards *et al,* 1995; Timmerman *et al,* 1995; Waldmeier *et al,* 1992) on basal GABA levels.

As a result of the limited response of GABA to TTX, Ca^{++} omission, and selective pharmacological agents, many investigators have questioned the neuronal origin of GABA in microdialysates. In this regard, it has been postulated that GABA measured by microdialysis may be derived from; non-classical neurotransmission, such as reversed-uptake by carrier mediated processes (Bernath & Zigmond, 1988; Pin & Bockaert, 1989); or from non-neuronal or cytoplasmic pools (Bernath *et al,* 1989), such as glial cells (Campbell *et al,* 1993), or elsewhere (Timmerman & Westerink, 1997).

Due to the discrepancies listed above, chromatographic conditions were examined to determine if they might have contributed to the results of GABA in the literature. It was concluded that a stringent pH of the mobile phase and a retention time of approximately 60 min is a prerequisite for a reliable HPLC separation of GABA. After optimisation of the HPLC conditions, animals were then exposed to a number of pharmacological challenges. A Ca^{++} and TTX sensitive GABA peak was detected, which responded strongly to infusion of various pharmacological agents, including baclofen.

Materials and Methods

Animals

Male albino rats of a Wistar-derived strain (300-350 g, Harlan, Zeist, The Netherlands) were used for the experiments. Animals were maintained on a standard 12-hour light/dark cycle at 24^{o}C and had access to food and water *ad libitum*.

Materials

Tetrodotoxin [TTX], nipecotic acid, baclofen, and phaclofen were purchased from Sigma-Aldrich Chemie B.V., The Netherlands. CGP 52432 was purchased from Tocris, USA.

Implantation and perfusion

Home made I-shaped microdialysis probes were constructed with a polyacrylonitrile/ sodium methyl sulfonate copolymer dialysis fibre (inner diameter 220 μm, outer diameter 310 μm; AN 69, Breda, The Netherlands). The dialysis probe was stereotactically implanted under the following conditions; isofluorane 2%: N_2O 300 mL/ min: O_2 300 mL/ min. Microdialysis probes were implanted at AP: -0.53, ML: +0.48, VD: -0.80 for hippocampus (4 mm dialysing membrane); AP: +0.09, ML: +0.30, VD: -0.60 for striatum (3 mm dialysing membrane); and AP: +0.33, ML: -0.08, VD: -0.60 for prefrontal cortex (3 mm dialysing membrane); according to coordinates from bregma (Paxinos and Watson, 1982). The microdialysis probes were permanently fixed to the skull using stainless steel screws and methylacrylic cement. Animals were allowed to recover 18-24 hours before microdialysis experiments commenced.

On the day of the experiment, the probes were perfused with Ringer solution containing NaCl 147.0 mM, KCl 3.0 mM, $MgCl_2.6H_20$ 1.0 mM, and $CaCl_2.2H_2O$ 1.2 mM. All pharmacological agents used for infusions were prepared in this Ringer solution, except experiments utilizing Ca^{++} free Ringer, or

high potassium Ringer solution where the ionic concentration was readjusted. Pharmacological challenges involving phaclofen, nipecotic acid and CGP 52432 were performed in hippocampus only.

In all experiments the dialysis probe was perfused at a rate of 1.5 μL/ min by TSE Univentor 802 syringe pump (TSE, Germany). Perfusion was allowed to stabilize for 120 minutes after which time dialysate samples were collected in 15-minute intervals. 7.5 μL of 0.02 M acetic acid were first added to the dialysate sample vials to prevent amino acid degradation. After six basal samples were collected, one-hour drug infusions were performed, followed by reperfusion of the Ringer solution. The collected samples were stored in a freezer at -80 ^{o}C.

At the end of the experiment, animals were anaesthetized with 1.0 mg/ kg sodiumpentobarbitol and then sacrificed. Coronal sections (16 μm thick) were made, and dialysis probe placement was localized according to the atlas of Paxinos and Watson (1982).

GABA analysis

GABA concentrations in the dialysates were determined off-line by pre-column derivatization with o-phtaldialdehyde/ mercaptoethanol reagent, and separation by reverse-phase HPLC in conjunction with fluorometric detection. The derivative reagent was prepared as follows, based on the derivitization by Lindroth and Mopper (1979). 100 mg o-phtaldialdehyde was dissolved in 2 mL methanol and added to 200 mL 0.5 mol/L $NaHCO_3$ (pH adjusted to 9.5 with NaOH) containing 20 μL 2-mercaptoethanol. The reagent was freshly prepared daily.

30 μL microdialysate samples were derivatized with 50 μL o-phtaldialdehyde/ mercaptoethanol reagent, mixed, and allowed to react for two minutes. 50 μL of the reaction mixture was then injected by a Gilson 401C autosampler (Gilson, France) onto the HPLC apparatus.

The derivatization mixture was separated using an isocratic mobile phase and measured by fluorometric detection. The mobile phase consisted of 70 mM di-sodium hydrogen phosphate, 400 μM EDTA, 0.15% (v/v) tetrahydrofurane, and a range of methanol concentrations. In order to determine the optimal organic modifier concentration, various mobile phases were made up with 50%, 45%, 40%, 35%, and 30% (v/v) methanol. Brain microdialysate quality samples were

measured under these conditions at different pH parameters, ranging from 6.00, 5.95, and so on decreasing by 0.05 pH units, to 4.50. The pH of the mobile phase was adjusted with phosphoric acid.

The HPLC system consisted of a Supercosil LC-18-DB column (150 X 4.6 mm, particle size 3 μm), a Gynkotec 300C high precision pump (flow rate 0.95, or 1.00 mL/ min) and a JASCO FP-1520 fluorometric detector (excitation λ = 350 nm, emission λ = 450 nm).

In vitro recovery experiments

The recovery of GABA across the dialysis membrane was determined by perfusing 1.2 mM Ca^{++} Ringer solution at a rate of 1.5 μL/ min through a 50 μL standard concentration of GABA placed in a beaker. Samples were collected every 20 minutes and determined as above for GABA concentration.

Expression of results and statistics

All values given are expressed as percentage of the average of 5 base line samples. The average concentration of five stable baseline samples was set at 100%. Statistical analysis (Sigmastat 3.0, 2003) was performed using one-way analysis of variance with repeated measures and Dunnett's multiple comparison test for post-hoc determination of significant differences. The level of significance was set at $p < 0.05$.

Results

All results are shown as the amount of femtomoles of GABA present, per microlitre of sample, per mm of membrane exposed to dialysis (fmol/ μL/ mm) followed by the standard error of the mean. The recovery of GABA across the dialysis membrane was determined as 23.35 ± 3.1%. Results were not corrected for *in vitro* probe recovery.

Modification of the mobile phase

Pooled microdialysate samples were collected and served as quality controls for GABA analysis. It was observed that by decreasing the methanol concentration, the GABA peak separated from various co-eluting peaks in the chromatogram (data not shown). Also, most of these peaks were larger than the relatively small amount of GABA. It was therefore decided to analyse the pooled samples using a large range of chromatographic conditions: pH's ranging from 6.00, 5.95, and so on decreasing by 0.05 pH units, to 4.50) at the various methanol concentrations (30%, 35%, 40%, 45%, 50%). A chromatogram in which GABA is clearly separated from co-eluting peaks is shown in Fig. 1. Fig. 2 illustrates that a pH change of 0.3 pH units moves the GABA peak over other unknown peaks of biological origin.

Fig. 1

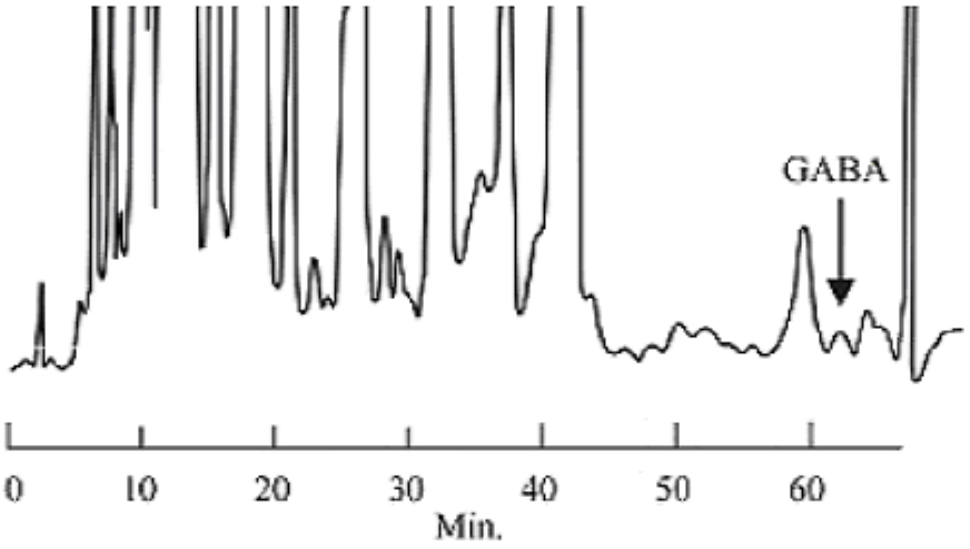

Figure 1 Typical chromatogram illustrating the elution point of GABA from pooled microdialysate samples at 30% methanol concentration, pH 5.25, and a flow rate of 0.95 mL/min.

Fig. 2

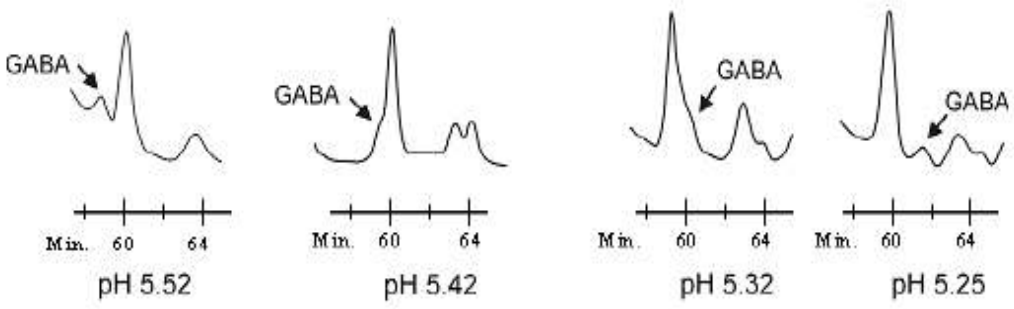

Figure 2 Effect of pH on GABA separation. A pH change of only 0.3 pH units moves the GABA peak over three other peaks of biological origin. HPLC was performed using mobile phase containing 30% methanol at a flow rate of 0.95 mL/min.

During the study it was observed that the retention time of GABA – in contrast to other amino acids – was extremely sensitive to the pH of the mobile phase (Fig. 3). It was concluded that stringent pH and methanol conditions, and a retention time of approximately 60 minutes are a prerequisite for the reliable separation of GABA. Moreover, even at a retention time of 60 min, only a small pH window was found (around 5.26 ± 0.01) in which GABA was consistently separated from co-eluting compounds.

Fig. 3

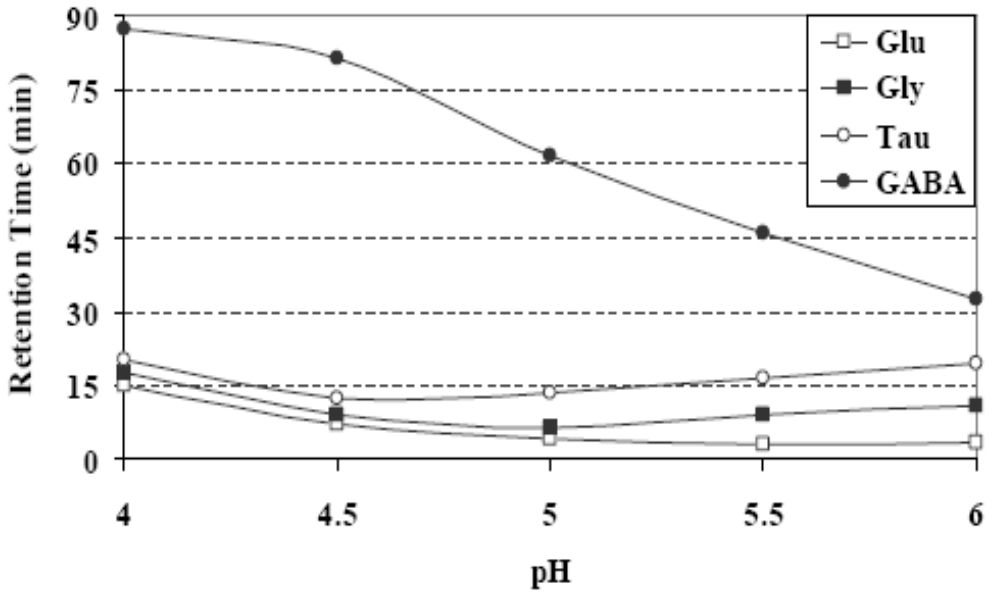

Figure 3 Effect of pH on the retention time of the amino acids glutamate, glycine, taurine, and GABA at a methanol concentration of 30% (v/v) in pooled hippocampal microdialysis samples; flow rate 1.0 mL/min.

The identity of the GABA peak was confirmed by injection of standards and by spiking the quality samples with GABA. The HPLC analysis of Ringer solution alone lacked the presence of any peaks near the retention time of GABA, which indicates that the co-eluting peaks observed in the pooled microdialysate samples are indeed of biological origin. We cannot confirm, however, that residual contamination of the signal does not exist, as there is no way to remove GABA from the dialysate samples to show that the peak disappears at zero concentration GABA.

Standard curve production and quality sample control

A standard curve was constructed, at the optimised mobile phase conditions, using concentrations ranging from 1.0 μM to 1.0 nM (Fig. 4), and was determined to be linear with a gradient of 0.995. Pooled microdialysis samples were determined as 3.11 ± 0.07 fmol/ μL/ mm membrane (n = 37).

Fig. 4

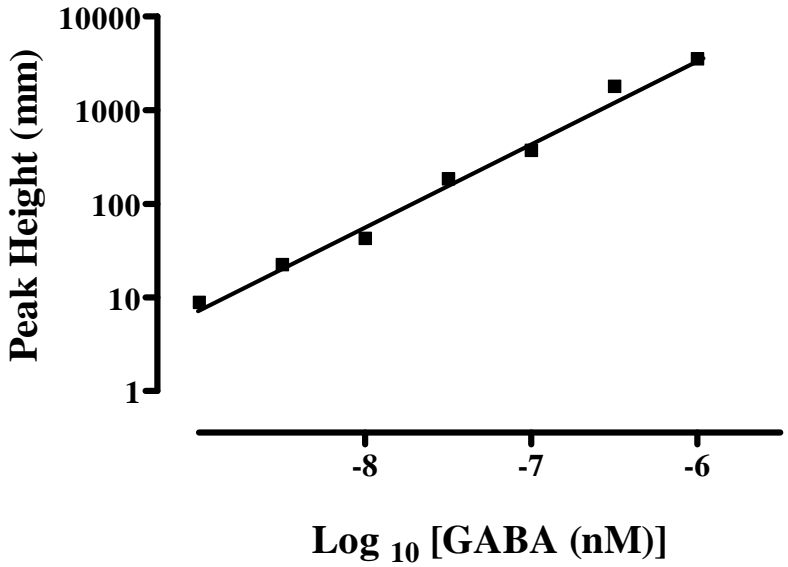

Figure 4 A standard curve illustrating the stability of the o-phtaldialdehyde derivative/ amino acid complex formed. The slope was determined as having a linear gradient of 0.995.

Effect of TTX and calcium-omission on extracellular levels of GABA

Basal GABA concentrations were determined as 2.45 ± 0.1, 2.95 ± 0.5, 3.62 ± 0.75 fmol/ µL sample/ mm dialysing membrane in hippocampus (n = 72), striatum (n = 26) and prefrontal cortex (n = 26) dialysate samples respectively.

It was determined that infusion of 1µM TTX significantly reduced GABA concentration by 60 ± 4.7%, 40 ± 5.8%, and 45 ± 4.8% in microdialysates in hippocampus ($\chi^2_9 = 39.872$), striatum ($\chi^2_8 = 31.641$), and prefrontal cortex ($\chi^2_8 = 32.184$) respectively (Fig. 5). 10µM TTX significantly reduced basal GABA levels by 65 ± 5.3%, 50 ± 6.6%, and 55 ± 3.1% in hippocampus ($\chi^2_8 = 37.782$), striatum ($\chi^2_8 = 29.387$) and prefrontal cortex ($\chi^2_8 = 31.644$) (Fig. 6). However, with both 1.0 µM and 10.0 µM TTX infusion, there was a considerable time delay (30 minutes) before a response to TTX was observed.

Fig. 5

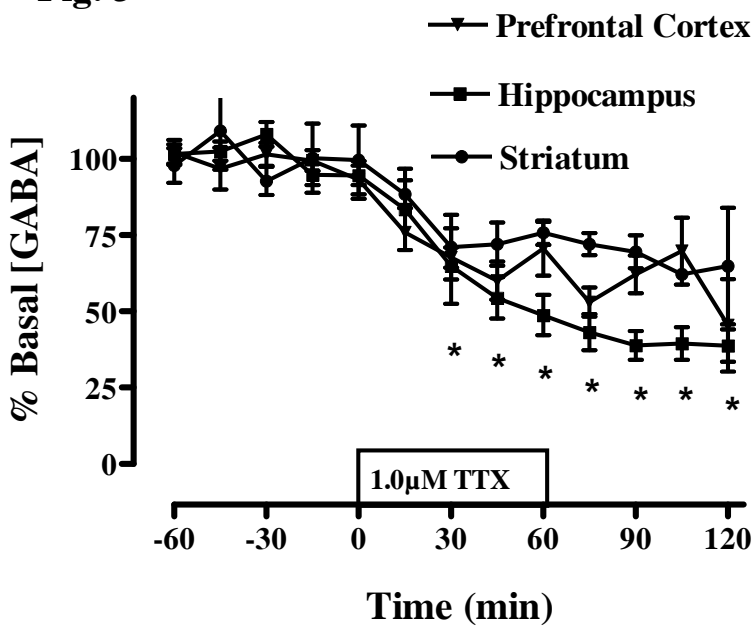

Figure 5 Time course of the effect of local infusion of 1.0 µM TTX on extracellular GABA in the hippocampus (n = 10), striatum (n = 8) and prefrontal cortex (n = 8). The solid horizontal bar indicates the period of infusion. Results are expressed as mean ± s.e.mean % change from pre-drug baseline levels. GABA levels were significantly decreased by the TTX infusion in microdialysate samples

from hippocampus (HC), striatum (STR) and prefrontal cortex (PFC) respectively (post hoc test indicates significance ($p < 0.05$: *)) from 30 -120 minutes after drug infusion.

Fig. 6

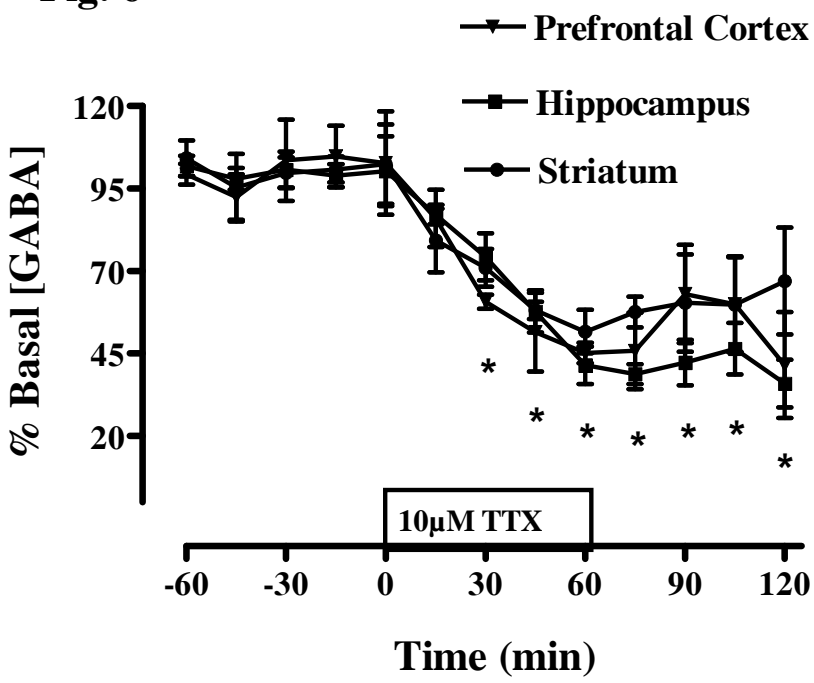

Figure 6 Time course of the effect of local infusion of 10.0 µM TTX on extracellular GABA in the hippocampus (n = 10), striatum (n = 8) and prefrontal cortex (n = 8). The solid horizontal bar indicates the period of infusion. Results are expressed as mean ± s.e.mean % change from pre-drug baseline levels. GABA levels were significantly decreased by the TTX infusion in microdialysate samples from hippocampus (HC), striatum (STR) and prefrontal cortex (PFC) respectively (post hoc test indicates significance ($p < 0.05$: *)) from 30 -120 minutes after drug infusion.

Following calcium omission, basal GABA concentrations were significantly reduced by 45 ± 6.3%, 33 ± 1.1% and 40 ± 4.8% in dialysates from hippocampus ($\chi^2_{10} = 33.200$), striatum ($\chi^2_{10} = 26.242$) and prefrontal cortex ($\chi^2_{10} = 23.697$) respectively (Fig. 7).

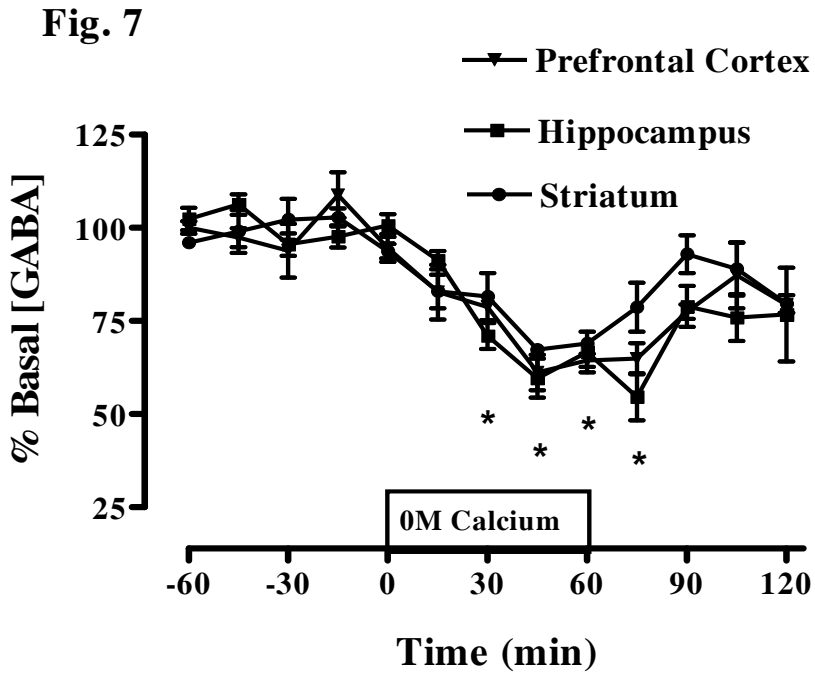

Figure 7 Time course of the effect of local infusion of 0M Ca^{++} Ringer solution on extracellular GABA in the hippocampus (n = 6), striatum (n = 5) and prefrontal cortex (n = 5). The solid horizontal bar indicates the period of infusion. Results are expressed as mean ± s.e.mean % change from pre-drug baseline levels. GABA levels were significantly decreased by 0M Ca^{++} infusion in microdialysate samples from hippocampus (HC), striatum (STR) and prefrontal cortex (PFC) respectively (post hoc test indicates significance ($p < 0.05$: *)) from 30 -90 minutes after drug infusion.

Effect of 100µM of the $GABA_B$ agonist baclofen, and the $GABA_B$ antagonists phaclofen, and CGP 52432.

The infusion of 100µM baclofen resulted in a dramatic 65 ± 2.9% reduction in hippocampal basal GABA levels ($\chi^2_9 = 33.498$), while there was a 50 ± 5.2% and 33 ± 2.8% reduction in prefrontal cortex ($\chi^2_9 = 36.422$) and striatum ($\chi^2_9 = 32.058$) respectively (Fig. 8).

Fig. 8

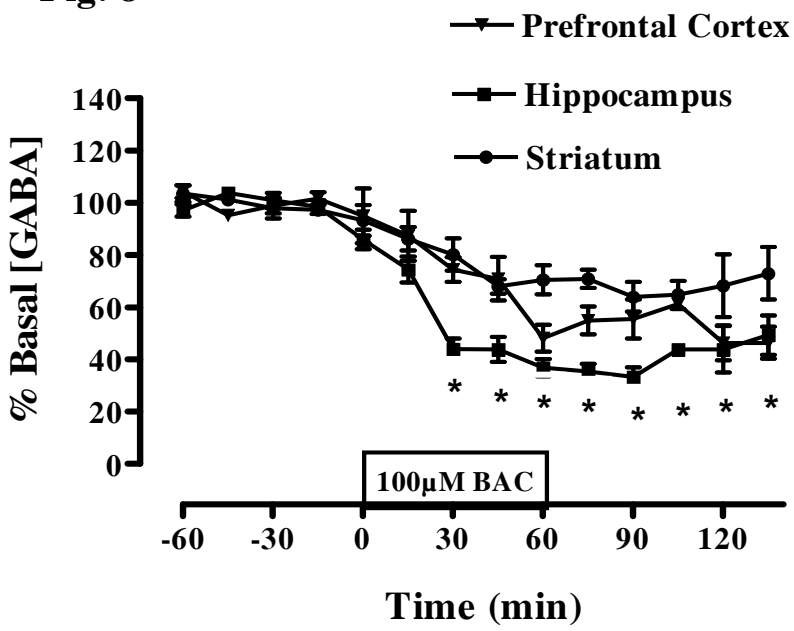

Figure 8 Time course of the effect of local infusion of 100µM baclofen on extracellular GABA in the hippocampus, striatum and prefrontal cortex. The solid horizontal bar indicates the period of infusion. Results are expressed as mean ± s.e.mean % change from pre-drug baseline levels. GABA levels were significantly decreased by baclofen infusion in microdialysate samples from hippocampus (HC), striatum (STR) and prefrontal cortex (PFC) respectively (post hoc test indicates significance ($p < 0.05$: *)) from 30 -150 minutes after drug infusion.

100µM of the $GABA_B$ antagonist phaclofen had no significant effect on basal GABA levels in hippocampus (Fig. 9). 100µM of the selective $GABA_B$ antagonist CGP 52432 produced a moderate yet significant 40 ± 3.3% increase in hippocampal basal GABA levels ($\chi^2_9 = 42.862$).

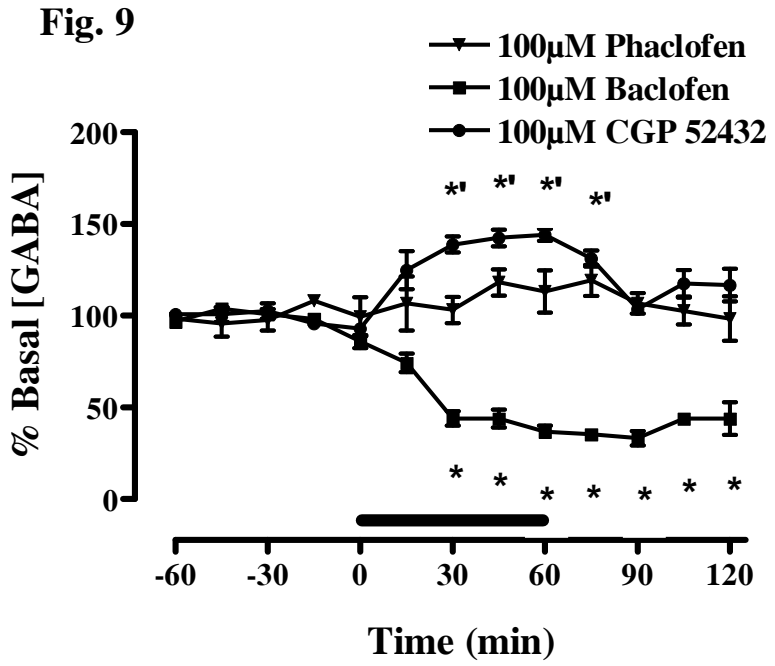

Figure 9 Time course of the effect of local infusion of the $GABA_B$ agonist baclofen (n = 6), and the $GABA_B$ antagonists phaclofen (n = 6) and CGP 52432 (n = 6) on extracellular GABA in the hippocampus. The solid horizontal bar indicates the period of infusion. Results are expressed as mean ± s.e.mean % change from pre-drug baseline levels. GABA levels were significantly decreased by the baclofen infusion (post hoc test indicates significance ($p < 0.05$: *)) from 30 - 150 minutes after drug infusion, and significantly increased by the CGP 52432 infusion (post hoc test indicates significance ($p < 0.05$: *´)) from 30 - 75 minutes after drug infusion, while phaclofen infusion had no significant effect on extracellular GABA levels.

Effect of elevated potassium, and nipecotic acid on extracellular GABA levels

1 hour local infusion of 60 mM K^+ caused a rapid 20-fold increase in basal GABA levels ($\chi^2_{10} = 34.246$), while the GABA reuptake inhibitor nipecotic acid, infused in concentrations of 50, 100 and 500 µM, caused a dose dependent increase in the basal GABA levels (χ^2_{10} = 13.184: 32.067: and 41.090) (Fig. 10). 500 µM

nipecotic acid yielded a 10-fold increase, which returned to normal levels after reperfusion with 1.2 mM Ca^{++} Ringer solution.

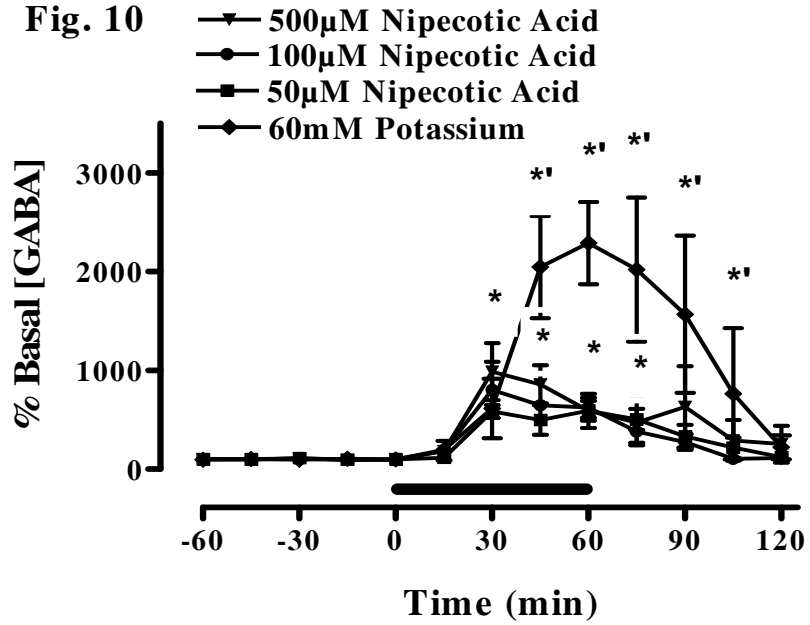

Figure 10 Time course of the effect of local infusion of 60mM K^+ (n = 7), and 3 different concentrations of nipecotic acid (n=7) on extracellular GABA in the hippocampus. The solid horizontal bar indicates the period of infusion. Results are expressed as mean ± s.e.mean % change from pre-drug baseline levels. After 30 minutes, GABA levels were significantly increased by high potassium infusion (post hoc test indicates significance from 30 - 105 minutes after drug infusion: $p <$ 0.01: *´), and all nipecotic acid infusions (post hoc test indicates significance from 30 - 105 minutes after drug infusion: $p < 0.05$: *).

Discussion

The chromatography of GABA

In the present study it was investigated whether the chromatographic separation of GABA in conjunction with fluorometric detection may have contributed to the controversial findings of numerous microdialysis studies on this neurotransmitter. To that end, we extended the chromatogram of the o-phtaldialdehyde derivatized samples by decreasing the methanol concentration in order to increase the retention time of GABA. When the retention time of GABA was increased to approximately 60 minutes, it was evident from the chromatograms that there were a number of peaks of biological origin that compromised the accurate detection of GABA. It appeared that the chromatographic conditions for optimal analysis of GABA were much more critical than described in the literature. The retention time of GABA seems to be very sensitive to the pH of the mobile phase, as compared with the retention times of other amino acids such as glutamate and glycine. After having analysed pooled microdialysis samples, using a large range of chromatographic conditions (pH's ranging from 6.00, 5.95, and so on decreasing by 0.05 pH units, to 4.50) at the various methanol concentrations (30%, 35%, 40%, 45%, 50%), it was concluded that stringent pH and methanol conditions are required. Moreover, a retention time of approximately one hour is a prerequisite for a reliable chromatographic separation of GABA. It was shown that a change in mobile phase as small as 0.3 pH units moved the GABA peak over other unknown biological peaks. It is emphasized that even with a retention time for GABA of 60 min, only a small pH window was found (around 5.26 ± 0.01) in which GABA was well separated from the co-eluting compounds. This illustrates that a precise and constant pH meter is also a prerequisite for appropriate analysis of GABA.

It has been discussed in the literature that the GABA-OPA derivative is unstable (Smith and Sharp, 1994). However, the linear standard curve obtained indicates that once injected onto the separation column, the GABA-OPA derivative is stable even at retention times as long as 60 minutes.

The identity of the GABA peak was confirmed by spiking with GABA standards. The fact that the standard peaks corresponded to the retention time of GABA, and that the selective pharmacological agent nipecotic acid dramatically altered the peak height reinforced the identity of this peak as the GABA peak. Basal GABA levels of 2.45 ± 0.1, 2.95 ± 0.5, 3.62 ± 0.75 fmol/ µL sample/ mm dialysing membrane were obtained in dialysates from hippocampus, striatum and prefrontal cortex respectively. These results are lower than many values reported in the literature.

The fact that the GABA peak is so sensitive to pH and methanol concentration, may account for some of the discrepancies in the literature. The high basal values, and the lack of response to various pharmacological challenges reported, may be due to peaks that co-elute with GABA, thus masking the true pharmacological effect.

Pharmacology of GABA Release

Under the modified chromatographic conditions, it was observed that TTX resulted in a 65 ± 5.3% (hippocampus), 50 ± 6.6% (striatum) and 55 ± 3.1% (prefrontal cortex) decrease in basal GABA levels. The omission of Ca^{++} caused a 45 ± 6.3%, 33 ± 1.1%, 40 ± 4.8% decrease in GABA basal levels in microdialysates from hippocampus, striatum and prefrontal cortex respectively. This indicates that a significant portion of GABA measured by the modified analytical approach is indeed of neuronal origin.

Elevated potassium levels (60 mM) resulted in a 20-fold increase in basal GABA levels, whereas the GABA uptake inhibitor nipecotic acid resulted in a dose-dependent increase in GABA. This nipecotic acid-induced 10-fold increase of GABA concentration is comparable to some reports in the literature (Campbell *et al,* 1993; Smith & Sharp, 1994; Westerink & De Vries, 1989) but much higher than other reports (Del Arco *et al,* 1998; Gallindo *et al,* 1999; Timmerman & Westerink, 1997; Timmerman *et al,* 1992; Voisin *et al,* 1994).

Another controversy in the literature is the inability to detect effects of $GABA_B$ autoreceptor agonists such as baclofen (Richards *et al,* 1995; Timmerman *et al,* 1995; Waldmeier *et al,* 1992). Interestingly, the present analytical approach clearly indicates that, similar to *in vitro* studies (Lambert & Wilson, 1994; Lanza

et al, 1993; Baumann *et al,* 1990, Waldmeier *et al,* 1988), infusion of baclofen decreased the GABA levels by 65 ± 2.9% in hippocampus. This decrease in GABA release is explained by a negative autoreceptor feed-back mechanism. The less potent $GABA_B$ antagonist phaclofen was not effective, but the potent compound CGP 52432 was shown to increase GABA levels by 40 ± 4.5% as compared with basal levels, indicating that a certain GABA inhibitory tonus is present in the hippocampus of control animals.

As the long retention time decreases the intensity of the GABA peak, the assay can only be reliably carried out by using a very sensitive fluorometric detection system. We were unable to reach this sensitivity using an electrochemical detector. Therefore the present results are only valid for fluorometric assays. However, as the discrepancies of microdialysis of GABA are both found for electrochemical and fluorometric assays, we believe that a more critical use of the electrochemical method is also justified.

The delayed response of GABA

When the time-curves in the present study are considered, there is a striking time delay before the onset of effects of TTX or calcium-omission, as compared with neurotransmitters such as dopamine which respond within 5 min (Feenstra and Botterblom, 1996). The delayed response may be explained by the efficient role that glial cells play in removing neuronally released GABA. Similarly to glutamate neurotransmission (Oliet *et al,* 2001), the GABA transporters of the glial cells might be concentrated near the synaptic cleft. It is likely that the microdialysis probe is in much closer contact with glial cells than with the synaptic cleft and this may result – in spite of the presence of GABA transporters – in a certain transport of GABA to the perfusion fluid. In this view, the glial cells may serve as a buffer between the GABA synapse and the microdialysis probes, which might explain the delayed response of GABA in the pharmacological experiments.

In conclusion, the present results illustrate that if the proper analytical conditions are applied, neuronal derived GABA can be sampled and quantitated by microdialysis in free-moving animals. However the proposed glial buffer between

the synaptic cleft and the microdialysis probe may prevent the detection of rapid changes of neurotransmission-derived GABA.

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